

Amendments to the Claims

Claim 1 (currently amended): A compound ~~of~~comprising Formula 1:



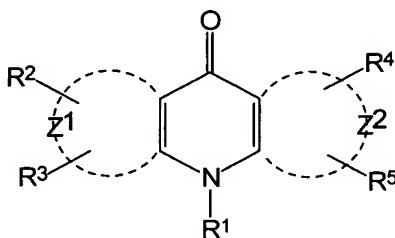
(I)

wherein R is a fluorescent dye molecule;

L is an optional linkage group containing one or more atoms comprising hydrocarbon chains which may also contain other atoms such as N, O and S; and

S is molecule comprising a substrate group of the enzyme aromatase ~~characterised in that~~ and further wherein the fluorescence signal of said compound changes in respect of fluorescence lifetime when the compound is acted upon by an enzyme with aromatase activity.

Claim 2 (currently amended): ~~A compound according to~~ The compound of claim 1, wherein said R is an acridone dye of Formula II:



(II)

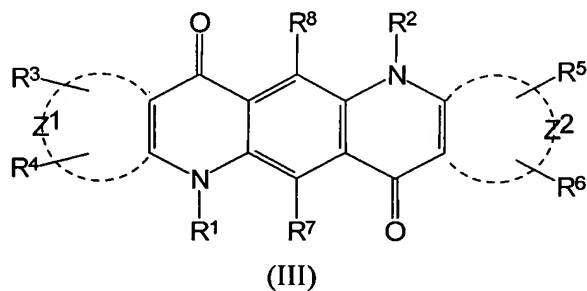
wherein:

groups R² and R³ are attached to the Z¹ ring structure and groups R⁴ and R⁵ are attached to the Z² ring structure;

Z^1 and Z^2 independently represent the atoms necessary to complete one or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;

R^1 , R^2 , R^3 , R^4 and R^5 are independently selected from hydrogen, halogen, amide, hydroxyl, cyano, amino, mono- or di- C_1 - C_4 alkyl-substituted amino, sulphhydryl, carbonyl, C_1 - C_6 alkoxy, aryl, heteroaryl, C_1 - C_{20} alkyl, aralkyl; the group -E-F where E is a spacer group having a chain from 1-60 atoms selected from the group consisting of carbon, nitrogen, oxygen, sulphur and phosphorus atoms and F is a target bonding group; and the group $-(CH_2)_nY$ where Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary ammonium and carboxyl and n is zero or an integer from 1 to 6.

Claim 3 (currently amended): ~~A compound according to~~ The compound of claim 1, wherein R is a quinacridone dye of Formula III:



wherein:

groups R^3 and R^4 are attached to the Z^1 ring structure and groups R^5 and R^6 are attached to the Z^2 ring structure;

Z^1 and Z^2 independently represent the atoms necessary to complete one or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected

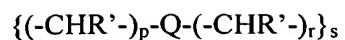
from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;
 R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 and R^8 are independently selected from hydrogen, halogen, amide, hydroxyl, cyano, amino, mono- or di- C_1 - C_4 alkyl-substituted amino, sulphydryl, carbonyl, carboxyl, C_1 - C_6 alkoxy, aryl, heteroaryl, C_1 - C_{20} alkyl, aralkyl; the group -E-F where E is a spacer group having a chain from 1-60 atoms selected from the group consisting of carbon, nitrogen, oxygen, sulphur and phosphorus atoms and F is a target bonding group; and the group $-(CH_2)_nY$ where Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary ammonium and carboxyl and n is zero or an integer from 1 to 6.

Claim 4 (currently amended): ~~A compound according to any of claims 1 to 3~~ The compound of claim 1, wherein L is a linker group containing from 1 to 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from $-NR'$ -, $-O$ -, $-S$ -, $-CH=CH$ -, $-C\equiv C$ -, $-CONH$ - and phenylenyl groups, wherein R' is selected from hydrogen and C_1 to C_4 alkyl.

Claim 5 (currently amended): ~~A compound according to any of claims 1 to 4~~ The compound of claim 1, wherein L is a linker group containing from 2 to 30 atoms.

Claim 6 (currently amended): ~~A compound according to any of claim 1 to 5~~ The compound of claim 1, wherein L is a linker group containing from 6 to 20 atoms.

Claim 7 (currently amended): ~~A compound according to any of claims 1 to 6~~ The compound of claim 1, wherein L is a linker group selected from the group:



where each Q is selected from CHR', NR', O, -CH=CH-, Ar and -CONH-;

each R' is independently hydrogen or C₁ to C₄ alkyl;

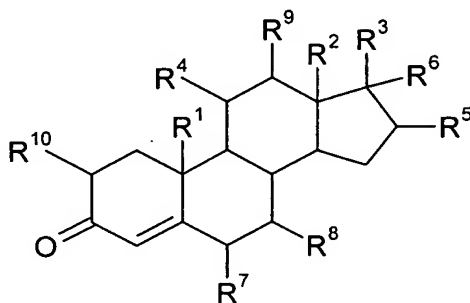
each p is independently 0 to 5;

each r is independently 0 to 5;

and s is either 1 or 2.

Claim 8 (currently amended): ~~A compound according to~~ The compound of claim 7,
wherein Q is selected from the group consisting of -CHR'-, -O- and -CONH-, where
R' is hydrogen or C₁ to C₄ alkyl.

Claim 9 (currently amended): ~~A compound according to any preceding claim~~ The
compound of claim 1, wherein S is a substrate group of the enzyme aromatase of
formula IX



(IX)

wherein:

R¹ and R² are selected from H and methyl;

R³ is selected from H, C₁-C₈ alkyl, cyano, -(CH₂)_k-OR^a;

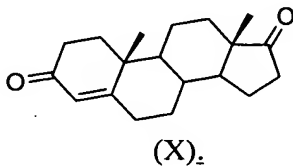
-(CH₂)_k-COOR^a; -(CH₂)_k-SO₃R^a; -(CH₂)_k-CHO, -(CH₂)_k-NR^bR^c and

-(CH₂)_k-COR^d;

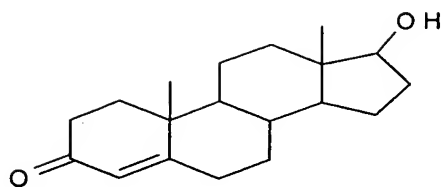
R^4 is selected from H, $-COR^a$ and hydroxyl;
 R^5 is selected from H, $-COR^a$, hydroxyl, cyano and halide;
 R^6 is selected from H and hydroxyl;
 R^7 , R^8 and R^9 are independently selected from H, $-COR^a$ and hydroxyl;
 R^{10} is selected from H and halide; and
 where R^a is selected from H and C1 – C4 alkyl, optionally substituted with OH; R^b
 and R^c are selected from H and C₁-C₄ alkyl;
 R^d is selected from C₁-C₈ alkyl or C₁-C₈ alkyl optionally substituted with COOR^a, OH,
 OR^a or SO₃R^a;
 and k is zero or an integer from 1 to 8.

Claim 10 (currently amended): ~~A compound according to~~ The compound of claim 9,
 wherein Group S is a steroid selected from the group of steroid families consisting of
 4-androsten-3-one, 4-cholesten-3-one, 4-estren-3-one and 4-pregnen-3-one
 derivatives.

Claim 11 (currently amended): ~~A compound according to any of claims 1 to 10~~ The
compound of claim 1, wherein S is androstenedione of Formula X or a derivative
 thereof:

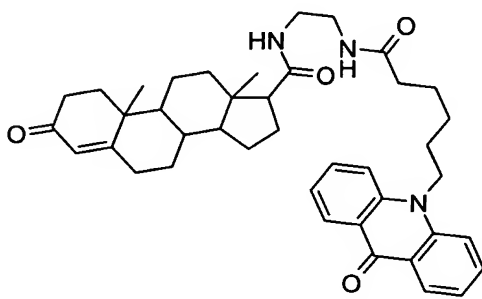


Claim 12 (currently amended): ~~A compound according to any of claims 1 to 10~~ The
compound of claim 1, wherein S is testosterone of Formula XI or a derivative thereof:



(XI).

Claim 13 (currently amended): ~~A compound according to any preceding claim of~~ The compound of claim 1, having Formula XX



(XX).

Claim 14 (currently amended): A method for measuring aromatase activity in a sample, the method comprising the steps of:

- i) measuring the fluorescence lifetime of ~~a compound according to any preceding claim~~ the compound of claim 1 prior to adding it to said sample;
- ii) adding said compound to said sample under conditions which favour aromatase activity, and
- iii) measuring a change in fluorescence lifetime of said compound following step ii);

wherein said change in fluorescence lifetime can be used to determine aromatase activity.

Claim 15 (currently amended): ~~A method according to~~ The method of claim 14, wherein the sample is selected from the group consisting of extract, cell, tissue and organism.

Claim 16 (currently amended): A method of screening for a test agent whose effect upon the activity of aromatase is to be determined, said method comprising the steps of:

- i) performing the method of claim 14 ~~or 15~~ in the presence of said agent; and
- ii) comparing the activity of said aromatase in the presence of the agent with a known value for the activity of aromatase in the absence of the agent;

wherein a difference between the activity of the aromatase in the presence of the agent and said known value in the absence of the agent is indicative of the effect of the test agent upon the activity of aromatase.

Claim 17 (currently amended): The method ~~according to~~ of claim 16, wherein the known value is stored ~~upon~~ in an electronic database.

Claim 18 (currently amended): A method of screening for a test agent whose effect upon the activity of aromatase is to be determined, said method comprising the steps of:

- i) performing the method of claim 16 ~~or 17~~ in the presence and in the absence of the agent; and

- ii) determining the activity of said enzyme in the presence and in the absence of the agent;

wherein a difference between the activity of aromatase in the presence and in the absence of the agent is indicative of the effect of the test agent upon the activity of aromatase.

Claim 19 (currently amended): The method ~~according to~~ of claim 17, wherein said difference in activity between the activity of aromatase in the absence and in the presence of the agent is normalised, stored electronically and compared with a value of a reference compound.

Claim 20 (currently amended): A method for measuring the distribution of a ~~compound of any of claims 1 to 13~~ the compound of claim 1 within a tissue, wherein the compound is capable of being taken up by a living cell within said tissue, the method comprising the steps of:

- i) measuring the fluorescence lifetime of the compound in a cell-free environment or a parental host cell;
- ii) adding the compound to one or more cells or a cell engineered to over-express aromatase, and
- iii) measuring the fluorescence lifetime of the compound following step ii);

wherein a change in fluorescence lifetime indicates aromatase activity and can be used to determine the distribution of the compound.

Claim 21 (currently amended): ~~A method according to~~ The method of claim 20,
wherein the distribution of the compound within the tissue of a first subject is
compared with the distribution of the compound within the tissue of a second subject.

Claim 22 (original): The method of claim 21, wherein said subject is selected from the
group consisting of mammal, plant, insect, fish, bird, fly, nematode and algae.

Claim 23 (original): The method of claim 22, wherein the mammal is a mouse or a rat.

Claim 24 (cancelled)

Claim 25 (currently amended): ~~A~~ In a method of diagnosing a disease caused by an
increase in aromatase activity in a subject, ~~using the method according to claim 14,~~
~~comprising the improvement comprising performing the method of claim 14, and~~
comparing the activity of aromatase in a sample taken from ~~a first~~ the subject with the
activity in a sample taken from a second healthy control subject, wherein any increase
in activity measured in the sample taken from the ~~first~~ subject relative to the second
healthy control subject is indicative of disease.

Claim 26 (currently amended): ~~Kit~~ A kit comprising:

- i) ~~a~~ the compound of claim 1 according to any of claims 1 to 13; and
- ii) an assay buffer; ~~and optionally~~
- iii) ~~a stop buffer.~~